Myeloproliferative Disorders

Introduction

This lesson is titled *Myeloproliferative Disorders*. Myelo, of the myeloid lineage, and proliferative, cancer making excess cells. In myeloproliferative disorders there is often an elevation of **all cell lines**—white blood cells, red blood cells, and platelets. Whichever cell line symptom predominates is what we name the disease. **Polycythemia vera** (PV) is the myeloproliferative disorder in which red blood cells predominate. **Essential thrombocytosis** (ET) is the myeloproliferative disorder in which platelets predominate. **Chronic myelogenous leukemia** (CML) is the myeloproliferative disorders in which the myeloid cell line predominates, usually neutrophils. **Primary myelofibrosis** (PMF) is a named disease, though we encourage you to learn it as the spent phase of PV and ET.

The myeloproliferative neoplasms (MPNs) are technically PV, ET, and CML. There is substantial overlap in the possible outcomes and each disease involves, in one way or another, the JAK/STAT pathway leading to proliferation. We want you to put a hard line in the sand and break the overlaps. CML is caused by the BCR-ABL fusion protein and will undergo a blast crisis to acute myelogenous leukemia. There is no other path. PV and ET have nothing to do with BCR-ABL, are caused by a JAK2 mutation (almost all PV, and half of ET) or an MPL mutation (the other half of ET), and progress to a burned out, hypoplastic, nonmalignant marrow.

Therefore, we want you to learn CML in the context of AML, in the context of leukemia, based on its progression to acute leukemia and its very different genetic mechanism of inducing cancer. But we cannot fully separate the discussion of BCR-ABL from the JAK2 system, so we'll use this lesson as an opportunity to build on what you already know.

Therefore, this lesson is about the physiology of the JAK2 system, the pathophysiology of BCR-ABL, how BCR-ABL is the same system as JAK2 but works differently, and the diseases polycythemia vera, essential thrombocytosis, and primary myelofibrosis.

More Details on the JAK2 System

JAK2 is a cytoplasmic kinase. When phosphorylated, JAK2 kinase is activated. Activated JAK2 kinase phosphorylates STAT. STAT goes to the nucleus and is a transcription factor that induces proliferation. JAK2 can become phosphorylated by a number of mechanisms, depending on what type of cell it is in. In this next discussion, the point is that JAK2 is activated by a receptor and receptor is a receptor tyrosine kinase. The only thing unique about each cell line is specifically which ligand induces dimerization and activates the kinase that activates JAK. Take a look at General Physiology#5: *Receptors* or glance at Figure 2.1.

The **erythropoietin receptor** is a receptor tyrosine kinase. It exists on committed erythroblasts. It is stimulated by its ligand, **erythropoietin** (EPO), inducing a dimerization and subsequent autophosphorylation of the erythropoietin receptor, activating the cytoplasmic kinase. That kinase phosphorylates JAK2.

The thrombopoietin receptor, named **MPL**, is a receptor tyrosine kinase. It exists on committed megakaryoblasts. It is stimulated by its ligand, **thrombopoietin** (TPO), inducing dimerization and subsequent phosphorylation of the thrombopoietin receptor, activating the cytoplasmic kinase. That kinase phosphorylates JAK2.

The G-CSF (granulocyte colony-stimulating factor) receptor is a receptor tyrosine kinase. It exists on committed myeloblasts. It is stimulated by its ligand, G-CSF, inducing dimerization and subsequent phosphorylation of the G-CSF-receptor, activating the cytoplasmic kinase. That kinase phosphorylates JAK2.

The GM-CSF (granulocyte-monocyte colony-stimulating factor) receptor is a receptor tyrosine kinase. It exists on common myeloid precursors. It is stimulated by the ligand GM-CSF, inducing dimerization and subsequent phosphorylation of the GM-CSF-receptor, activating the cytoplasmic kinase. That kinase phosphorylates JAK2.

See where I'm going with this? They are all receptor tyrosine kinases. Which one is expressed is a product of how differentiated the progenitor is. That's the only difference. And an undifferentiated progenitor can become any cell line, while a committed blast can only become that cell line.

BCR-ABL is a fusion gene created by a translocation of chromosomes 9 and 22—t(9;22)—the Philadelphia chromosome. **BCR-ABL is a cytoplasmic tyrosine kinase.** BCR acts like the receptor portion of a receptor tyrosine kinase in that it dimerizes and autophosphorylates the BCR-ABL protein, activating the ABL kinase activity. But unlike a receptor, the BCR-portions autoassociate and activate ABL without any signal—just one BCR-ABL protein being next to another activates both. **ABL is a kinase**. What ABL phosphorylates is . . . get ready for it . . . JAK2.

The other myeloproliferative disorders have various mutations in the pathway. Polycythemia vera, for example, is always caused by a JAK2 mutation that renders it constitutively activated, even without being phosphorylated. Essential thrombocytosis can also be caused by a JAK2 mutation, or it can be caused by a mutation in MPL, with constitutive activity of the thrombopoietin receptor's kinase activity. We aren't sure exactly how, but some mutations have been found in calreticulin as well. The point is, though, CML is t(9;22), the others are not.

DISEASE	MUTATION	CELL LINE
CML	BCR-ABL	Granulocytes
PV	JAK2	Erythrocytes
ET	JAK2, MPL, calreticulin	Megakaryocytes
PMF	JAK2, MPL, calreticulin	Probably burned out PV or ET

Table 2.1: Diseases of Myeloproliferation

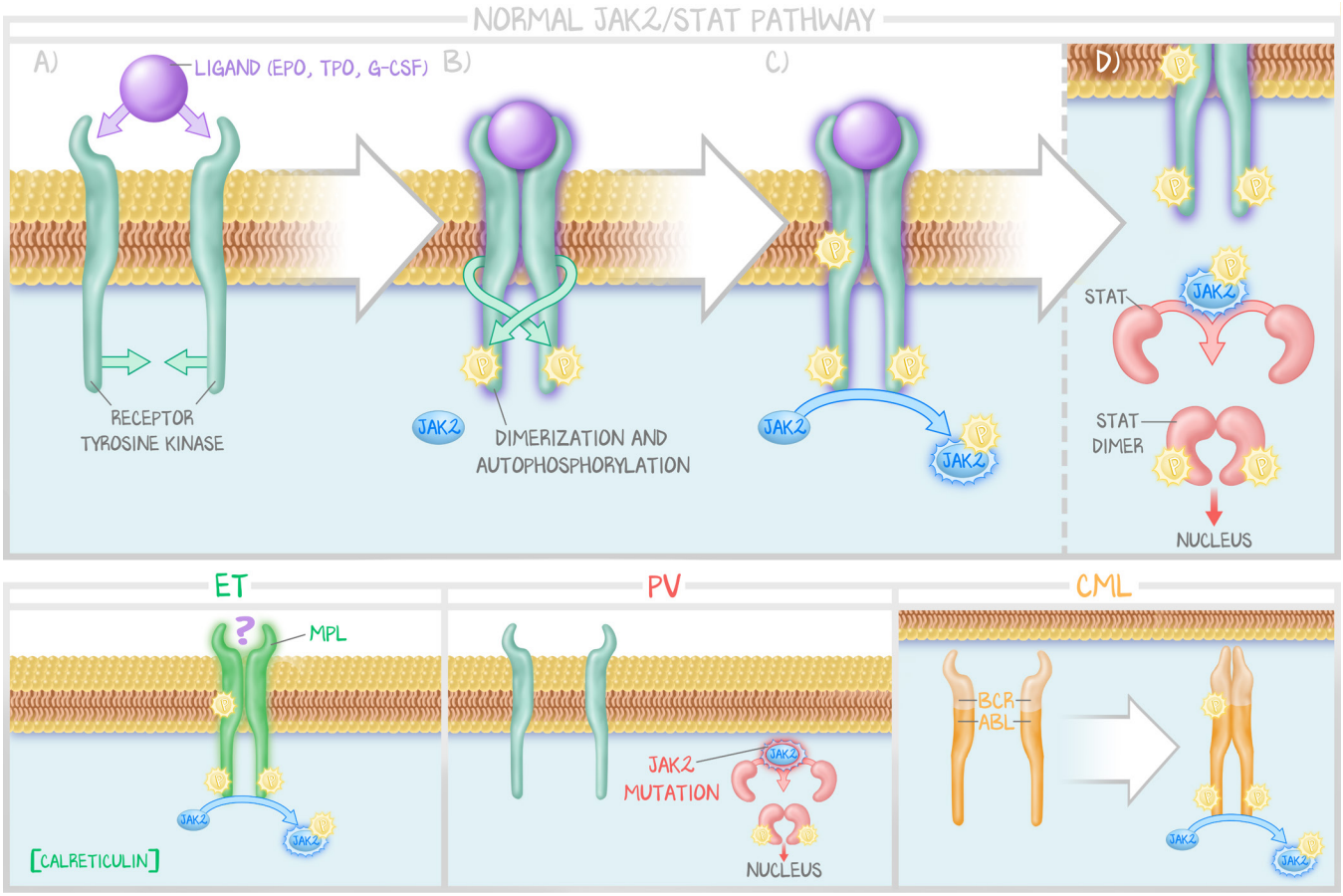


Figure 2.1: Mechanism of Action and Disease
The normal JAK/STAT pathway provides a connection between extracellular trophic signals and intracellular gene transcription. This is a growth signal triggered by the binding and dimerization of a receptor tyrosine kinase. In various disease states—constitutively active MPL, constitutively active JAK2, or a BCR-ABL fusion protein—something other than the binding of a trophic signal to its receptor leads to JAK2 kinase activity and subsequent STAT-induced gene transcription.

Destiny of Myeloproliferative Disorders

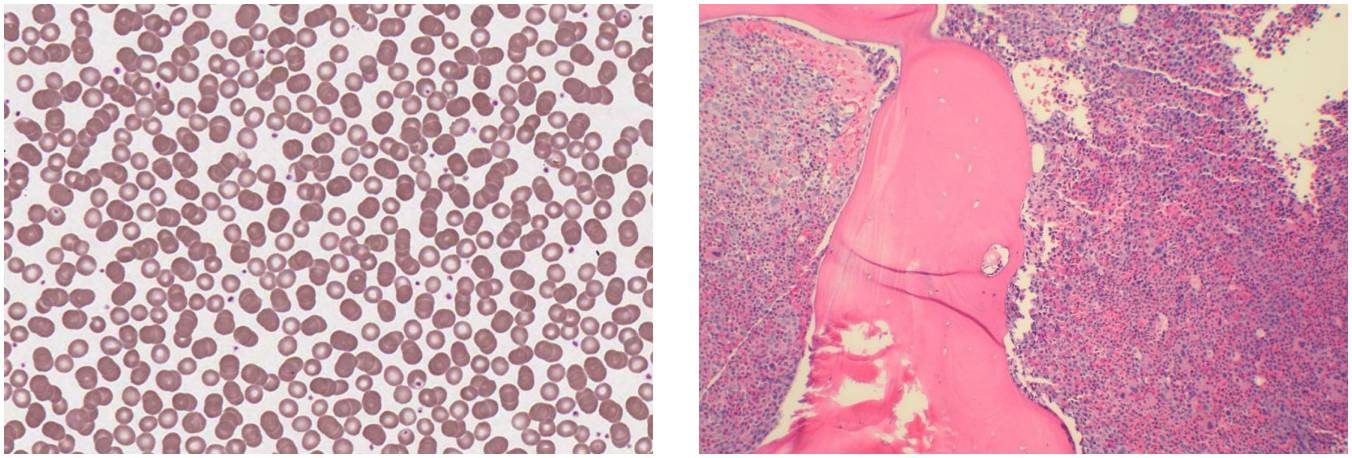
If a cancer cell replicates enough times, one of two things happens. The cell either develops telomerase and becomes immortalized, on its way to malignancy, or it reaches its maximum replications and either dies (telomere crisis) or enters senescence. There are only a small number of hematopoietic stem cells in the marrow. They provide the common progenitors, which in turn provide the blasts. CML progresses through myelodysplasia to leukemia. PV and ET progress to myelofibrosis.

If the early progenitor cells have the grow-unregulated signal but do not develop the immortalization, they die off. If they die off, there are no cells left to fill the marrow. In this path, the myeloproliferative disorder takes the path through myelofibrosis, emphasis on fibrosis. The marrow will be **hypocellular** and replaced by **collagen** (fibrosis). In **myelofibrosis** the hematopoietic centers shift to the extramedullary sites, in which leukemias may develop, though myelofibrosis tends to not become leukemia in the marrow. PV and ET tend to result in myelofibrosis and not malignancy. PV and ET are caused by JAK2, MPL, or calreticulin mutations. "Primary myelofibrosis" is also "caused by" JAK2, MPL, or calreticulin mutations. To us, this sounds like it is just burned out marrow.

If those progenitor cells have the grow-unregulated signal and DO develop immortalization, they replicate forever. They do not die off. The marrow is hypercellular, rich with malignant cells. With repeated replication cycles, more mutations are acquired. Without treatment, the eventuality is a dedifferentiation of the cancer. That dedifferentiation starts with only a small number of dysplastic cells. As they crowd out the marrow, more of the marrow is taken up by malignancy. Chronic leukemia is the production of mature myelocytes. But if enough mutations occur, if the dedifferentiation becomes severe enough, they become blasts, immature myelocytes. This is called **blast crisis**, and the cancer becomes acute myelogenous leukemia. CML results in blast crisis. We'll finish up the discussion of BCR-ABL-induced CML-to-AML blast crisis in Proliferation #3: *Leukemia*.

Polycythemia Vera

Of all the myeloproliferative disorders, polycythemia vera is the one most closely associated with JAK2 mutations—as in 99.99% of cases. Ninety-seven percent are associated with a valine-to-phenylalanine substitution at residue 617. The other 3% involve some other mutation of JAK2. The receptor tyrosine kinase that normally activates JAK2 is stimulated by a ligand. When the progenitor is an erythroblast, that receptor tyrosine kinase uses erythropoietin as its ligand. EPO stimulates the cell to divide and differentiate, acting as the growth signal. In JAK2 mutations, in which no stimulation from the receptor is needed, unregulated proliferation ensues. When the affected cell is an erythroblast, the red blood cells increase more than the other cell lines. PV increases red blood cells **without an erythropoietin stimulus**. In PV, the hemoglobin is elevated but **EPO is low**.



(a) (b)

Figure 2.2: Polycythemia Vera
(a) A blood smear of PV, denoting excess red blood cells and rouleuax formation. (b) The histology of bone marrow showing excess proliferating islands of erythroblasts.

The marrow of PV is hypercellular with little adipose. The increase in red cell progenitors is subtle and usually accompanied by an increase in granulocytes and megakaryocytes as well. All cell lines increase, but symptoms are because of increased red cell mass, volume, and hyperviscosity. The increase in red cell mass and increased volume of blood causes abnormal blood flow, particularly in the low-pressure venous side of circulation, in which veins become engorged. Patients are **plethoric** (they swell with fluid) and become **cyanotic** (the slow-moving blood allowing for oxygen to be extracted from hemoglobin). Patients complain of **intense pruritis**, especially in hot water (as in showering), thought to be caused by release of histamine from basophils. **Basophilia** is most pronounced in PV. The most dangerous complication of the disease is **thrombosis**. Because of the stagnant flow of blood, patients often come to the attention of medical care because of a **venous thrombosis**. However, because the disease also

demonstrates significant increase in platelets, **arterial thrombosis** is possible as well. The thing is, when a patient has a heart attack, stroke, or DVT, medical care tends to get a CBC. In PV, the CBC is wildly deranged—white count 12–50, hemoglobin 14–28, and platelets > 500.

Without intervention, death from thrombosis happens in months. With intervention, the patient lives for a decade or longer. Treatment is **phlebotomy**—keeping the hemoglobin and platelets at near-normal level is enough to avoid complications. PV rarely progresses to myelodysplasia, and instead progresses to a **spent phase** characterized primarily by **myelofibrosis**. Unlike CML, which can become ALL or AML, PV only progresses to AML, consistent with the cell of origin being a progenitor committed to myeloid differentiation—the erythroblast dedifferentiates "one tier up" to common myeloid precursor. See "Essential Thrombocytosis" (below) for more on "one tier up."

Reactive Polycythemia

An elevated Hgb does NOT necessarily mean a myeloproliferative disorder, does not necessitate that there is a mutation of an erythrocyte progenitor in the bone marrow. Instead, there may be an appropriate increase in proliferation of the marrow in response to an inappropriately elevated erythropoietin.

EPO drives red blood cell proliferation. If EPO is high, more red blood cells will be made. EPO is supposed to be elevated when the kidney feels hypoxia. The kidneys sense hypoxemia and tell the marrow to make more red blood cells. But where does the oxygen come from? The lungs. This is how the system works for healthy humans. But unhealthy humans with **chronic hypoxic lung disease** will provide the kidney with hypoxia, a drive to make more hemoglobin. The problem with that, of course, is that more hemoglobin won't fix the problem with the lung. And so, a reactive polycythemia, an elevated Hgb because of an elevated EPO, is seen in chronic diseases of the lung that provoke hypoxemia—COPD, pulmonary fibrosis, and the like.

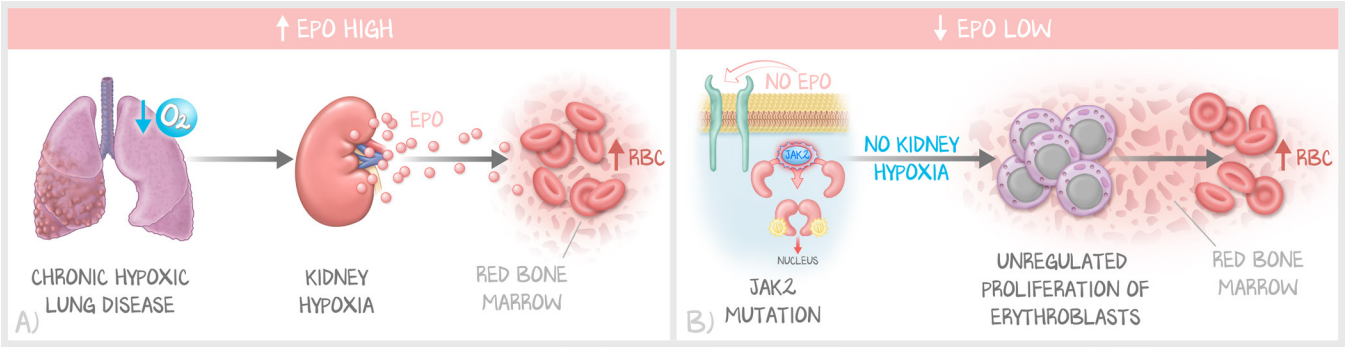


Figure 2.3: Polycythemia Vera vs. Reactive Polycythemia
(a) When there is some non-marrow related pathology that impairs renal oxygenation (usually chronic hypoxemic lung disease), the kidney induces excess production of red blood cells by increasing erythropoietin. RBCs are up because EPO is up. (b) When there is some marrow-related pathology that instigates excess red blood cells, the kidney turns off the EPO signal. RBCs are up so EPO is down.

Any condition that lowers the circulating oxygen can induce EPO production, too. **High altitude** is commonly cited as a driver for raising EPO levels. It's not that the kidney senses a lower barometric pressure around the person the kidney is in. But it does sense the lower partial pressure of oxygen in the air that person is breathing. That is to say, less oxygen in the lung has the same effect as hypoxemic lung disease—the blood doesn't have enough oxygen, and the kidney can tell. If a patient lived at high altitude but wore supplemental oxygen, the EPO would not rise.

The kidney is the source of the hormone EPO. **Renal cell carcinoma** is abnormal proliferation of cells that possess the ability to release EPO. One potential presentation of renal cell carcinoma is a **paraneoplastic syndrome**, where, without the stimulation of hypoxia, cancer cells secrete EPO anyway, thereby inducing a polycythemia.

In all of these conditions listed—hypoxic lung disease, high altitude, renal cell carcinoma—the hemoglobin is high **because the EPO is high**. In PV, the hemoglobin is high despite the EPO's being low.

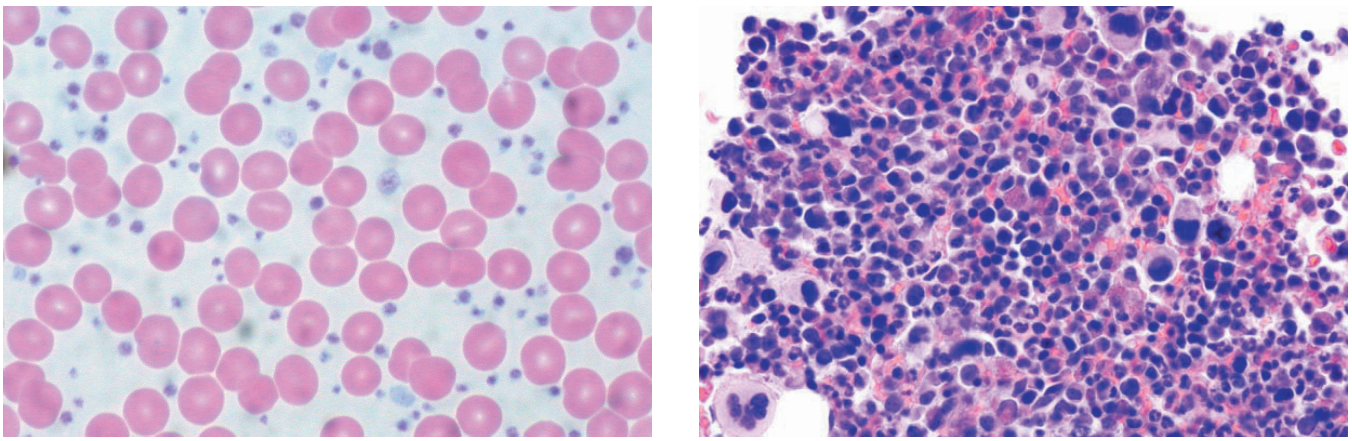
Essential Thrombocytosis

ET is caused by activating point mutations in JAK2 (just like PV, but only 50% of ET cases have the JAK2 mutation), MPL (the TPO receptor), or calreticulin. The connection between the JAK/STAT pathway and **calreticulin** has yet to be elucidated, and makes for a good "gotcha" test question. The connection between JAK/STAT and MPL is well understood. See if this sounds familiar.

MPL is the receptor tyrosine kinase that normally activates JAK2. It is stimulated by a ligand. When the progenitor is a megakaryoblast, that receptor tyrosine kinase uses thrombopoietin (TPO) as its ligand. TPO stimulates the cell to divide and differentiate, acting as the growth signal. With either MPL mutations or JAK2 mutations, no stimulation by TPO is needed, and unregulated proliferation ensues. When the affected cell is a megakaryoblast, the platelets increase more than the other cell lines. ET increases red blood cells **without a thrombopoietin stimulus.** Because the presence of platelets inhibits the release of TPO, in ET the platelets are elevated but **TPO is low**. Very similar to the description of PV.

When JAK2 mutations are present, it is the same point mutation as in PV. Why some patients develop ET and others PV is not understood. We explain it with the "one tier up" concept. If the JAK2 mutation occurs in a progenitor committed to platelets, it makes mostly platelets. If it dedifferentiates into the common myeloid precursor, all of the cells it had already made up to that point are committed to megakaryoblasts. Subsequent cells can become anything myeloid. Its just that the mutation happened first in a committed blast, so more cancer cells are platelet-committed.

Bone marrow cellularity is usually only mildly increased, but megakaryocytes are often markedly increased in number and include abnormally large forms. Peripheral smears usually reveal abnormally large platelets often accompanied by mild leukocytosis. Modest degrees of extramedullary hematopoiesis may occur, producing mild organomegaly in about 50% of patients.



(a) (b)

Figure 2.4: Essential Thrombocytosis
(a) Peripheral blood smear shows marked thrombocytosis, including some giant platelets that are almost the size of a red blood cell. (b) a bone marrow demonstrating excess proliferation and activity of megakaryocytes.

Dysfunctions of platelets derived from the neoplastic clone can lead to both thrombosis and hemorrhage, the major clinical manifestations. Platelets are not only increased in number, but also frequently demonstrate qualitative abnormalities in functional tests. The types of thrombotic events resemble those observed in PV; they include deep venous thrombosis, portal and hepatic vein thrombosis, and myocardial infarction.

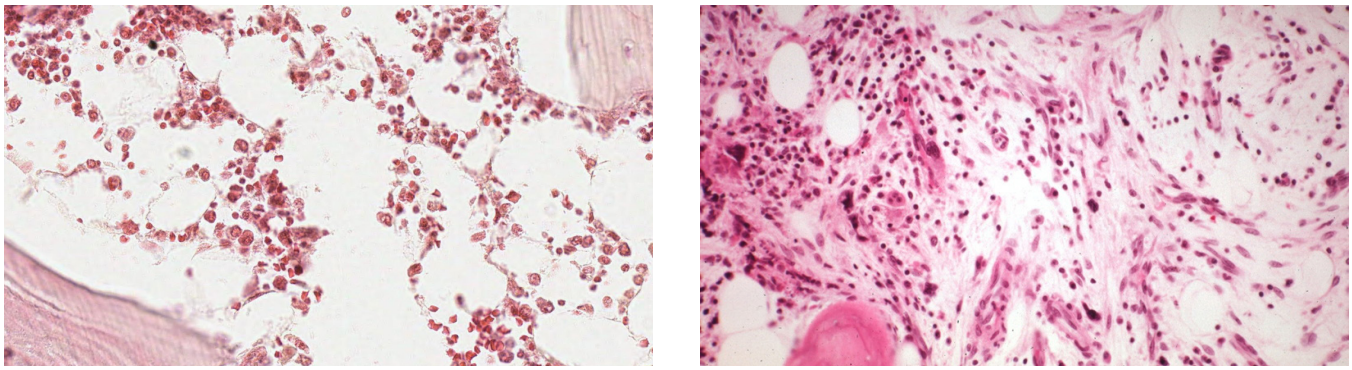
If there are fewer than a million platelets and there is thrombosis, use aspirin. If there are more than a million platelets, the risk of bleeding increases as the number indicates accumulation of platelet function errors, and aspirin is not indicated.

Myelofibrosis

This diagnosis is listed as a separate entity in pathology textbooks. All cases are found to have JAK2, MPL, or calreticulin mutations. We want you thinking about this disease as the spent phase of PV or ET, the burned-out marrow of a proliferation syndrome that did not accumulate telomerase. Because the marrow is spent, **fibrosis replaces the marrow**. It is not hypocellular with adipose (aplastic anemia); it is hypocellular because it has been replaced by fibrosis, with collagen. Because the marrow is burned out, **cell lines decrease**. The peripheral blood findings are nonspecific, and only a bone marrow biopsy can confirm.

Threats to life include intercurrent infections, thrombotic episodes, bleeding related to platelet abnormalities, and transformation to AML, which occurs in 5% to 20% of cases. When at the myelofibrosis phase, **extramedullary hematopoiesis** takes over. This induction of splenic and hepatic hematopoiesis is an abnormal process, activating embryonic stem cells in hematopoietic centers of embryogenesis. Activating abnormal processes may induce cancer, which is where the AML comes from. The fibrosed marrow rarely progresses to AML (< 5%), but myelofibrosis may provoke AML in extramedullary sites. The marrow is fibrotic, and while myelodysplasia may return in the marrow, leukemia is more likely to occur in an extramedullary site. The **JAK2 inhibitors** have been approved to treat myelofibrosis. It makes sense that they could also be used to treat PV and ET to prevent myelofibrosis.

Cytoreductive agents include JAK2 inhibitors, interferon-α, and hydroxyurea. Know they exist. They should be used in symptomatic PV or ET, in an attempt to prevent myelofibrosis.



(a) (b)

Figure 2.5: Aplastic Anemia vs. Myelofibrosis
(a) Bone marrow aspirate of aplastic anemia, without fibrosis. (b) Myelofibrosis bone marrow biopsy.